

EVIDENCE FOR SYMBIOTIC ALGAE IN SPONGES FROM TEMPERATE COASTAL REEFS IN NEW SOUTH WALES, AUSTRALIA

D.E. ROBERTS, S.P. CUMMINS, A.R. DAVIS AND C. PANGWAY

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The symbiotic relationships between tropical reef sponges and cyanobacteria (microalgae) has been well documented. Preliminary evidence suggests that these relationships may be just as common in temperate reef species. Screening of sponges from temperate reefs in New South Wales, Australia, found 5 out of 8 species tested were 'chlorophyll positive'. Of those tested, *Cymbastela concentrica* (Lendenfeld) had the greatest concentration of chlorophyll-*a* pigment within its tissue ($139.4 \pm 9.4 \mu\text{g/g}$). An estimate of the percentage of temperate reef sponges that potentially contained symbiotic algae was made based on their in situ colour pigmentation. It was predicted that over 65% of temperate reef sponges potentially contain symbiotic algae, although it is unknown how many may be phototrophic. □ *Porifera, symbiotic algae, cyanobacteria, temperate sponges, ecology.*

D.E. Roberts* (email: Dannio@bigpond.com.au), Department of Biological Sciences, University of Wollongong, Wollongong 2522, NSW; S.P. Cummins**, Water Science Section, Environment Protection Authority, Locked Bag 1502, Bankstown 2200, NSW; A.R. Davis, Department of Biological Sciences, University of Wollongong, Wollongong 2522, NSW; C. Pangway, Analytical Chemistry Laboratory, Environment Protection Authority, PO Box 29, Lidcombe 2141, NSW, Australia; Present address: *Wyang Shire Council, PO Box 20, Wyong 2259; **Wyang Shire Council, PO Box 20, Wyong 2259; 27 January 1999.

Many sponges from tropical reefs contain and benefit from symbiotic relationships with cyanobacteria or microalgae (Sarà, 1971; Vacelet & Donadey, 1977; Wilkinson, 1978, 1981, 1983; Larkum et al., 1988). Wilkinson (1983) suggested that in some species these algae can provide a substantial source of nutrition for the host sponge (see also Wilkinson, 1981; Wilkinson et al., 1988). In tropical regions, light levels may limit the maximum depth to which phototrophic sponges are distributed. Photosynthetic symbionts are unlikely to provide nutrition beyond the net photosynthetic compensation point (Cheshire & Wilkinson, 1991). In striking contrast, symbiotic algae may even provide photo-protection to some species of sponges from excessive light in shallow water (Sarà, 1971; Wilkinson, 1983).

Until recently, phototrophic sponges were thought to be restricted to sub-tropical and shallow tropical reef habitats (Wilkinson, 1983), however a temperate reef phototrophic sponge (*Cymbastela* sp.) is now known to be an exception (Cheshire et al., 1995). At least one tropical sponge with symbiotic algae, *Cymbastela concentrica* (Wilkinson, 1983; Larkum et al., 1988; Hooper & Bergquist, 1992; Hooper & Lévi, 1994), also has a temperate range where it is

found between 10-60m depth or more (Roberts & Davis, 1996).

We predicted that symbiotic relationships between temperate reef sponges and algae may be widespread because of the number of species exhibiting a colour, indicative of microalgae within their tissue (Hooper & Bergquist, 1992). Here we report the results of a screening study where eight common temperate reef sponges were tested for the presence of symbiotic algae. Furthermore, we extrapolated from our records on pigmentation for over 100 temperate species to gain some estimate of the potential for temperate reef sponges to contain symbiotic algae.

MATERIALS AND METHODS

To test for general evidence of symbiosis in temperate reef sponges we sampled a range of common species, some of which were replicated to estimate variation and consistency in both colour and chlorophyll concentration. Eight species of Demospongiae (Table 1) were haphazardly collected from the subtidal reefs at Henry Head and Inscription Point at the entrance to Botany Bay, New South Wales (NSW) Australia (Fig. 1), and the concentration of chlorophyll-*a* within their tissue was determined.

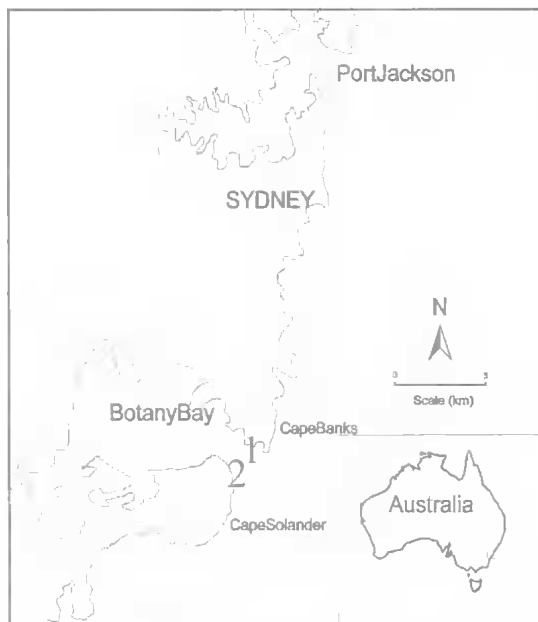


FIG. 1. Location of reefs at Inscription Point and Henry Head, NSW, where sponges were collected to determine the presence of symbiotic algae (1=Henry Head; 2= Inscription Point).

The collections were made on sponge-dominated reefs between 10-20m depth. Other common sessile macrobenthic organisms found on these reefs included ascidians, bryozoans, cnidarians, foliose macroalgae and a nondescript matrix of microorganisms and silt (Davis et al., 1997). Spatial and temporal patterns of abundance for these assemblages have been described elsewhere (Davis et al., 1997).

The sponges were photographed, their in situ colour recorded, brought to the surface, field weighed (to the nearest gram), and immediately transported back to the laboratory under dark conditions in fresh seawater. Variation in the concentration of chlorophyll-*a* within a species was also examined for two of the eight sponges, by collecting five replicate specimens of each of *C. concentrica* and *Clathria striata*. All sponges were identified and were included in a voucher collection lodged with the Queensland Museum.

To determine whether symbiotic algae were present in each of the sponges, 1g of tissue was removed and processed with 90% aqueous acetone solution. A sub-sample of 1g was found to give a suitable chlorophyll-*a* absorbance reading for a variety of sponge samples. To ensure the complete extraction of the chlorophyll-*a* pigment, the sponge tissue was mechanically broken down

using a mortar and pestle. The resultant extracted slurry was transferred to a screw-cap bottle and the total volume was adjusted to a constant volume (10ml) and stored at -20°C until analysis.

Immediately before spectrophotometric determination samples were removed from the freezer and returned to room temperature in the dark. The sample extracts were filtered through a solvent-resistant disposable filter (Whatman GF/C 47mm diameter), and 3ml of the solution was transferred to a 1cm cuvette. Absorbance was measured on a Cary 1E UV-visible spectrophotometer at 664nm for chlorophyll-*a*, and 750nm, 647nm and 630nm for turbidity, chlorophyll-*b* and chlorophyll-*c* corrections, respectively. Before sample analysis, the spectrophotometer absorbance was adjusted to zero by inserting a 90% acetone blank. The concentration of chlorophyll-*a* was calculated using the methods described in Clesteri (1985). It should be noted that the samples were not subsequently acidified and analysed for pheophytin-*a* (a common degradation product of chlorophyll-*a*). All work with chlorophyll extracts was undertaken in cool, dark conditions to minimise potential degradation of chlorophyll.

The in situ colour of a sponge may be a good preliminary test or indicator for the presence of symbiotic algae (Hooper & Bergquist, 1992). To estimate the percentage of temperate reef sponges that potentially contained symbiotic algae we examined our earlier collections of over 100 temperate reef species from Sydney to Port Stephens, NSW (Roberts & Davis, 1997; Roberts et al., 1998). Subjective estimates were made on the presence or absence of symbiotic algae based on the in situ colour of each species from field records and photographs.

RESULTS

Five of the eight species of temperate reef sponges we tested (in four orders and seven families) were found to be 'chlorophyll positive'. These were *Clathria striata*, *Phoriospongia cf. kirki*, *Cymbastela concentrica*, *Callyspongia* sp. and *Spirastrella areolata* (Table 1). Of the five species, *C. concentrica* had the greatest concentration of chlorophyll-*a* within its tissue, followed by *Callyspongia* sp. (Table 1). Little within-species variation was found in the mean concentration of chlorophyll-*a* (\pm S.E.) in the tissue of *C. concentrica* (139.4 ± 9.4) and *C. striata* (19.3 ± 1.4) (Table 1).

The other three common species tested were also chlorophyll positive (Table 1), although

TABLE 1. Summary of sponges from temperate reefs (10-20m depth) screened for the presence of symbiotic algae (* n = 5 sponges).

Order	Family	Species	Chlorophyll-a ($\mu\text{g/g}$)
Hadromerida	Spirastrellidae	<i>Spirastrella areolata</i> Dendy	20.7
Halichondrida	Axinellidae	<i>Cymbastela concentrica</i> (Lendenfeld)	139.4 \pm 9.4 *
Haplosclerida	Callyspongiidae	<i>Callyspongia</i> sp.	85.3
Poecilosclerida	Tedaniidae	<i>Tedania digitata</i> (Schmidt)	Nil
	Microcionidae	<i>Clathria striata</i> Whitelegge	19.3 \pm 1.4 *
		<i>Holopsamma arborea</i> (Lendenfeld)	Nil
	Phoriospongiidae	<i>Phoriospongia</i> cf. <i>kirki</i> (Bowerbank)	10.2
Raspailiidae	<i>Ceratopsion aurantiaca</i> (Lendenfeld)	Nil	

chlorophyll-*a* concentrations were considerably lower than in *C. concentrica* and *Callyspongia* sp. *Spirastrella areolata* is a massive ridge shaped sponge, which has an olive-yellow colour, whilst *C. striata* is a fan-shaped, orange-tan coloured sponge. *Phoriospongia* cf. *kirki* grows as a massive- ridged shaped form and is characteristically a cream-tan colour throughout. It had the lowest measurable concentration of chlorophyll-*a* and contained small dark-brown nodules along the side of each ridge within the ectosomal outer layer. We are not certain whether the chlorophyll positive result we obtained for this species was due to these dark-brown nodes and further work would be required to identify the distribution of symbiotic algae within this species.

Other species *T. digitata*, *C. aurantiaca* and *H. arborea* all returned a 'chlorophyll negative' response for the presence of symbiotic algae (Table 1). Both *T. digitata* and *C. aurantiaca* were bright orange in colour, which probably reflects metabolically produced carotenoid pigments within the sponge (Hooper, 1996). These pigments may be photo-protective and have been predominately observed in the Poecilosclerida and Axinellida (Hooper, 1996). *Holopsamma arborea*, a common white, honey-combed reticulated sponge (Hooper, 1996) on shallow and deep reefs in NSW (Roberts & Davis, 1996) was also chlorophyll negative.

Four orders of sponges examined in this preliminary study had chlorophyll positive species (Table 1). Wilkinson (1993) also found chlorophyll positive species in each of 5 orders he examined from tropical reefs (see Table 2). Over 100 species of temperate reef sponges were examined for their potential to contain symbiotic

algae based on their in situ colour. It was estimated that of these species, 65% potentially contained symbiotic algae.

DISCUSSION

Our findings show that over 60% of sampled temperate reef sponges have chlorophyll levels consistent with the presence of microalgae. Of the 5 species which we identified as being 'chlorophyll positive', we expected *C. concentrica* and *Callyspongia* sp. to contain chlorophyll-*a*, based entirely on the colour of their external tissue. Since *Cymbastela* spp. were reported as phototrophic in temperate and tropical waters (Cheshire et al., 1995; Wilkinson, 1983; Larkum et al., 1988), it was highly likely that this would be the case for related species in NSW.

Wilkinson (1983) found that nine out of ten of the most common sponges on a tropical reef (representatives from five orders and six families) contained symbiotic algae. It has been estimated that up to 50% of tropical sponges may rely on this symbiosis because of the relatively low levels of available nutrients in these waters (Cheshire & Wilkinson, 1991). It is our opinion that a large proportion of shallow water temperate reef sponges may also have these associations, given that five of the eight species we screened were 'chlorophyll positive'. Furthermore, in our subjective examination of over 100 species from these temperate waters, at least 65% had similar colour to those that proved to be chlorophyll positive in this preliminary analysis.

On shallow water temperate reefs, *C. concentrica* was found to have on average 139.4 \pm 9.4 $\mu\text{g/g}$ of chlorophyll-*a* within its tissue. Wilkinson (1983) reported a concentration of 93.4 $\mu\text{g/g}$ of chlorophyll-*a* in *C. concentrica* (described as *Pseudaxinyssa* sp. in his work) from a tropical reef. Seddon et al. (1993) investigated the ability of *C. concentrica* to photoacclimate in shallow water, and suggested that factors other than visible light were important in restricting its distribution. A preliminary study by Cheshire et al. (1995) on a *Cymbastela* sp. from southern Australian waters (possibly *C. notiana* Hooper & Bergquist), demonstrated that these sponges were capable of maintaining themselves

TABLE 2. Sponge orders found to be chlorophyll positive on temperate versus tropical reefs (nt = not tested).

Sponge Order	Temperate (this study)	Tropical (Wilkinson, 1983)
Astrophorida	nt	1/1
Dictyoceratida	nt	5/6
Hadromerida	1/1	nt
Halichondrida	1/1	1/1
Haplosclerida	1/1	nt
Poecilosclerida	2/5	1/1
Clathrinida	nt	1/1

photosynthetically. Given the results we have obtained in this study, we anticipate that *C. concentrica* and many other temperate reef sponges may have this ability. Whether or not temperate reef sponges rely on symbionts to enhance nutrition is unknown, however the work by Cheshire et al. (1995) in South Australia would suggest that this is the case.

Cymbastela concentrica is typically an olive-brown colour (Hooper & Bergquist, 1992) at its surface, but variations in the shade of colour have been observed between shallow and deep waters. This may indicate changes in the concentration of symbiotic algae within the sponge associated with light gradients. Initial experiments with *C. concentrica* suggest that its colour can 'lighten' within days of manipulating its position with respect to incident light. *Cymbastela concentrica* and a number of other temperate reef sponges have been shown to be adversely affected by the discharge of sewage effluent into shallow and deep-water habitats (Roberts, 1996; Roberts et al., 1998). We speculate that any symbiotic relationship between sponges and algae may be altered through reductions in available light and/or increased nutrients as a result of sewage effluent (Roberts et al., 1998).

We believe that many temperate reef sponges contain symbiotic algae however the significance of any symbiosis has to be quantified. We need to identify the types of symbionts within various species and examine these relationships with light gradients. Although temperate reef sponges may contain symbiotic algae, their role in the nutrition of the sponge needs to be quantified.

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NEW COLONIAL VACELETIA-TYPE SPHINCTOZOAN FROM THE PACIFIC.

Memoirs of the Queensland Museum 44: 498. 1999:- Three new morphotypes of a Recent colonial sphinctozoan coralline sponge are presented. All types show close relationships to the taxon *Vaceletia crypta*, a non-colonial form from Indo-Pacific reef caves. The first two types were discovered in shallow water reef caves of Osprey Reef, N Queensland Plateau in the Coral Sea. These sponges are common in these caves. The third type of colonial sphinctozoan was found only at two localities at North Astrolabe Reef and Great Astrolabe Reef in Fiji. This variety shows similarities with a previously described deep water variation of *Vaceletia* from New Caledonia.

The first two morphotypes of colonial *Vaceletia* from Osprey Reef show more similarities to the cryptic, non-colonial form *V. crypta* from reef caves of the Great Barrier Reef and reefs of the Indo-Pacific, than to the deep-water colonial species described by Vacelet (1988) and Vacelet et al. (1992) from New Caledonia. The third variation from Astrolabe Reef is more similar to this deep water variation from New Caledonia. All three variations will be described elsewhere in detail as multidisciplinary taxonomic and geochemical investigations of these taxa are still in progress (Reitner & Wörheide, 1995; Wörheide & Reitner, 1996).

The discovery of these three new colonial variations from shallow water reef caves of the SW Pacific clearly demonstrates that colonial forms of Recent *Vaceletia* are not restricted to deep waters, as previously thought.

Sphinctozoan sponges were primary reef building organisms during the Permo-Triassic. They are chambered calcified sponges with morphological similarities to Cambrian Archaeocyaths. The *Vaceletia*-type of coralline sponges occurred first in the middle/late Triassic (Reitner, 1992). Sphinctozoans were considered to be rare since the end of the Triassic, and were thought to be extinct since the end of the Cretaceous; that is until the 'living fossil' *Vaceletia* was discovered by Vacelet (1979) in the Indian Ocean.

The solitary, non-colonial form *Vaceletia crypta* has no reef building potential and is found only sparsely dispersed in the darker areas of Indo-Pacific reef caves.

These recently discovered colonial variations of *Vaceletia* from shallow water reef caves retain a colonial growth mode and a reef building capability.

They provide, therefore, clues to understand the modalities of skeletal construction and biocalcification, as well as the ecology of Permo-Triassic sphinctozoan sponges. □ *Porifera, coralline sponges, mud-mounds, Vaceletia, colonial reef-building sphinctozoans, Osprey Reef, Coral Sea.*

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Joachim Reitner (email: jreitne@gwdg.de) & Gert Wörheide*, Institut und Museum für Geologie und Paläontologie, Universität Göttingen, Goldschmidt-Strasse 3, D-37077 Göttingen, Germany; John N.A. Hooper, *Queensland Museum, P.O. Box 3300, South Brisbane, Qld. 4101, Australia; 1 June 1998.